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Effect of excipients on acetaminophen metabolism and its implications for prevention of liver injury

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Abstract

Acetaminophen poisoning is the most frequent cause of acute hepatic failure in the US. Toxicity requires reductive metabolism of acetaminophen, primarily via CYP2E1. Liquid acetaminophen preparations contain propylene glycol, a common excipient that has been shown to reduce hepatocellular injury in vitro and in rodents. Children are less susceptible to acetaminophen toxicity for unclear reasons. We conducted a pharmacokinetic single-blinded crossover study of 15 healthy adult volunteers comparing the CYP2E1 and conjugative metabolism of a 15mg/kg dose of liquid versus solid preparations of acetaminophen. Measured AUC's for the CYP2E1 metabolites were 16-17% lower and extrapolated AUC's were 25-28% lower in the liquid formulation arm while there was no difference in conjugative metabolite production. The formation rate constants for reductive metabolites were equivalent between solid and liquid formulations indicating that enzyme inhibition was competitive. Propylene glycol, an established CYP2E1 competitive antagonist, was detected in the liquid formulation but not solid formulation arm. Since children tend to ingest liquid preparations, the protective effect of this excipient could

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explain their decreased susceptibility to acetaminophen toxicity. A less hepatotoxic formulation of acetaminophen could potentially be developed if co-formulated with a CYP2E1 inhibitor.

Keywords

Acetaminophen; propylene glycol; liver failure; CYP2E1

Introduction

Acetaminophen (APAP) poisoning is the leading cause of acute hepatic failure in the United States (U.S.) and Europe ^{1,2}. An estimated 33,520 patients are hospitalized in the U.S. following APAP poisoning annually.³ APAP poisoning is responsible for half of the liver transplants that follow drug-induced liver failure in the U.S. ⁴ Cases of hepatic failure due to APAP toxicity continue to rise.^{1,5}

Hepatocellular injury is initiated by the metabolism of excess amounts of APAP. Most of the APAP ingested is metabolized by direct phase II conjugation with sulfate and glucuronide. Toxicity is due to an increase in the amount of APAP undergoing reductive metabolism, primarily via cytochrome P450 2E1 (CYP2E1), and can occur following intentional overdose or during chronic supratherapeutic dosing.⁶ Most clinicians and investigators believe that hepatocellular damage is mediated via N-acetyl-p-benzoquinone imine (NAPQI), the principal reactive metabolite produced by CYP2E1 (Figure 1A).

Recent investigation into the epidemiology of APAP poisoning reveals that cough and cold preparations, which are often liquid formulations, are underrepresented among APAP formulations causing toxicity.⁵ Additionally, children appear less susceptible to APAP-induced liver injury than adults.⁷ Suggested mechanisms include increased sulfonation capacity, relatively larger liver size as well as increased relative supply of glutathione.⁸⁻¹² However, children tend to ingest liquid preparations instead of solid and we believe it is the preparation and not the unique physiology of children that could explain this protective benefit.

Liquid APAP preparations contain propylene glycol (PG), a solubilizing agent used to dissolve APAP in aqueous solutions.¹³ In murine and in vitro models of acetaminophen toxicity, PG has been found to reduce hepatic injury via inhibition of CYP2E1.^{14,15} We therefore sought to investigate the difference in metabolism following ingestion of liquid versus solid APAP preparations in adults to see if an excipient may be conferring a hepatoprotective effect.

Methods

Participants

Subjects were recruited via internet advertisement. Eligibility criteria included age 18-40 years, no daily medications and no chronic or acute medical conditions. Specific exclusion criteria were any history of liver disease, ingestion of two or more alcoholic beverages more than four times per week, or pregnancy.

Study Protocol

The protocol and all interventions were approved by the Institutional Review Board at the Beth Israel Deaconess Medical Center. We performed a single-blinded crossover pharmacokinetic study at our institution's clinical research center.

Each subject had a screening visit to review medical history and obtain consent. Baseline testing included serum chemistries, renal function tests, liver function tests (Roche Hitachi Modular), complete blood counts (Sysmex XE2100) and a urine pregnancy test.

Subjects had two separate study visits at least one week apart to allow for full clearance of APAP metabolites. They were instructed not to take any APAP or ethanol for 48 hours prior to each visit. Subjects were randomized to receive a standard commercially available tablet or liquid formulation of APAP on the first visit. The alternative preparation was given on the second visit. At presentation for each visit, plasma APAP concentrations were measured to confirm lack of use. Subjects underwent a one hour fast, then received a 15mg/kg oral dose of APAP. The liquid dose was always equivalent to the closest possible rounded-down solid dose that could be administered with standard APAP tablets. Plasma samples were collected at 30 minutes, and at 1, 2, 4, 6 and 8 hours after APAP administration. Subjects were provided a standard lunch.

Blood samples were refrigerated for not more than 24 hours and then plasma was separated by centrifugation. All samples were then stored at -80° C.

Quantitative Assays of Acetaminophen and Metabolites

The authors (MB, DW) performing the LC/MS/MS analysis were blinded to the formulation administered at each visit. Plasma samples were assayed for APAP, APAP-glucuronide, APAP-sulfate (conjugative metabolites), APAP-cysteinate and APAP-mecapturate (reductive metabolites). Samples were prepared by diluting one volume of plasma with two volumes of acetonitrile, vortexing for ten seconds, then placing the mixture on ice for ten minutes. This was followed by centrifuging samples for six minutes at 14,000 rpm, diluting with the initial mobile phase, and centrifuging for an additional two minutes before transferring final solutions into limited-volume (250 µL) autosampler vials for 5 µL injections into the HPLC system. Samples were analyzed with an Agilent 1100 HPLC system coupled to an AB/SCIEX API 3000 mass spectrometer. Analytes were separated on a Kinetex C18 column (100 × 2.1 mm, 2.6 µm particles, Phenomenex, Torrance, CA), using mobile phases of water (A) and acetonitrile (B), each containing 0.1% formic acid, with gradient elution as follows: 8% B from 0 to 1.2 min, to 70 % B at 6 min, to 85% B at 7 min, to 95% B at 10 min, returned to 8% B at 10.2 min and equilibrated to 20 minutes before the next injection. Standards for calibration were prepared by spiking minimal volumes of concentrated solutions of each standard (1 mg/mL of APAP-cysteinate, APAP-glutathione and APAP-mercapturate and 10 mg/mL of APAP-glucuronide, APAP-sulfate and APAP) into plasma that was collected from a healthy volunteer taking no medications that had been screened to establish that these compounds were absent.

Quantitative Assays of Propylene Glycol

Plasma PG concentrations were measured at NMS Labs using a Hewlett Packard 5890 GC with FID detector. Samples from time points zero through 120 minutes from three subjects were assayed first. PG was detected in the 30-minute time point in all three, and in one subject at 120 minutes. Given these initial results and since enteral absorption of PG is rapid, subsequently all of the 30 minute time point samples from all subjects were assayed. The limit of detection of this assay is 5.0 mg/dL.

Pharmacokinetic and Statistical Evaluation

Power analysis was based on the following assumptions: we expected approximately five to ten percent of the ingested APAP to undergo reductive metabolism.¹⁶⁻¹⁸ Assuming a difference of 50% between groups, a calculated sample size of ten subjects would exceed the number needed to enroll with a power of at least 80%. To allow for attrition, we planned to enroll 15 subjects.

Area under curve to the last time point (AUC_0^t) was calculated using the trapezoidal method and individual profiles were also fitted by nonlinear regression. Initial pharmacokinetic parameter estimates were determined for each subject by fitting the concentration data separately, then optimized by simultaneously fitting all data using NONMEM 7.2 software (ICON, Dublin Ireland). AUC's extrapolated to infinity (AUC_0^∞) and formation rate constants for all metabolites (k_s , k_g , k_c , k_m) were thus calculated (based on the compartmental model in Figure 1B). Peak concentrations, AUC's, percent metabolites and formation rate constants of liquid versus solid preparations were all compared with a paired-sample 2-tailed T-test.

Results

A total of 15 subjects completed both study visits. The mean age of the subjects was 28 years, 10 (67%) subjects were male, and their mean weight was 82kg (range 60-128 kg). The mean acetaminophen dose administered was 1197 mg (range 825-1900mg, median dose 1150mg) and 9 (60%) subjects were randomized to start with the liquid formulation.

There was no statistically significant difference in measured and modeled AUC's or peak concentrations for APAP and the phase-II metabolites (APAP-glucuronide and APAP-sulfate) following ingestion of solid vs. liquid preparations (Table 1 and Figures 2A, 2B). There was a trend towards greater bioavailability of the liquid formulation, however this was not significant (APAP AUC_0^t 54.5 vs 51.4 $\mu\text{g}\cdot\text{hr}/\text{mL}$, $p=0.089$).

Following ingestion of the liquid preparation (relative to the solid) there was 17% less measured APAP-cysteinate (AUC_0^t 2.411 vs. 2.009 $\mu\text{g}\cdot\text{hr}/\text{mL}$, $p=0.007$), 25% less modeled APAP-cysteinate (AUC_0^∞ 5.699 vs. 4.288 $\mu\text{g}\cdot\text{hr}/\text{mL}$, $p=0.007$), 16% less measured APAP-mercaptopurine (AUC_0^t 0.716 vs. 0.602 $\mu\text{g}\cdot\text{hr}/\text{mL}$, $p=0.006$) and 28% less modeled APAP-mercaptopurine (AUC_0^∞ 1.515 vs. 1.096 $\mu\text{g}\cdot\text{hr}/\text{mL}$, $p=0.005$) in serum. The peak concentrations were also significantly lower following liquid formulation ingestion for both APAP-cysteinate and APAP-mercaptopurine (Table 1 and Figure 2C).

The goodness of fit achieved in the nonlinear regressions of the metabolite measurements resulted in a range of coefficients of correlation between 0.932 and 0.986, standardized residuals with no detectable trends and mostly within -3 and +3, and predictive confidence regions encompassing all observations with minimal over- and under-prediction.

There was no significant difference in formation rate constants for APAP-cysteinate, APAP-mercapturate or APAP-sulfate (Figure 3) The formation rate constant for APAP-glucuronide was significantly greater for the solid as compared to liquid formulation (0.777 vs. 0.622 1/hr, $p=0.015$), however there was large overlap between the interquartile ranges.

PG was detected in 9 out of 15 30-minutes samples obtained following ingestion of the liquid preparation, but in none of the samples in the solid group. The average PG concentration in these 9 samples was 6.2 mg/dL and the maximum concentration was 9.1 mg/dL.

Discussion

APAP is bioactivated to NAPQI, the radical metabolite generally considered to be the necessary initiating step in APAP-induced liver injury, primarily via CYP2E1. Intracellular glutathione is the major hepatocyte redox buffer; it binds NAPQI producing APAP-glutathione, which is subsequently metabolized to the cysteinate and mercapturate metabolites.^{19,20} We show a 16-28% decrease in production of the CYP2E1 derived metabolites in plasma following administration of a commercially available liquid preparation thus supporting our hypothesis that a drug vehicle present in the liquid but not solid formulation is inhibiting the bioactivating enzymes. Previous cell culture and animal experiments and our formation rate constant data suggest, but does not prove, the PG is the excipient responsible for the effect we observed.

APAP is only sparingly soluble in aqueous solutions and consequently solubilizing agents, including PG, are necessary in liquid preparations. PG is a known competitive inhibitor of CYP2E1.²¹ Given that bioactivation via the P450 system is necessary for APAP toxicity, the effect of PG on APAP toxicity has been studied in primary hepatocyte culture as well as in rodents.^{14,15,21} Cultured hepatocytes were less susceptible to APAP-induced necrosis in the presence of PG.²¹ Mice pretreated with PG but not fluvoxamine, a CYP1A2 inhibitor, showed reduced hepatocellular damage following a toxic APAP dose.¹⁵ In a mouse model, the concept that drug vehicles are protective of APAP-hepatotoxicity has been studied: dimethylsulfoxide, dimethylformamide, PG, and ethanol were shown to be protective of APAP-induced liver injury.¹⁴ Additionally, compared to wild-type mice, CYP2E1 knock-out mice demonstrate minimal increases in liver transaminases after an LD₅₀ dose of acetaminophen; however, at this higher dose, CYP1A2 and CYP3A may contribute to NAPQI formation.²²

Drug-drug interactions affecting APAP toxicity and potential manipulation of the P450 system in order to alter APAP toxicity in humans is not a new concept. Subjects who received 2 months of cimetidine, an established P450 inhibitor, were noted to have decreased urinary APAP-mercapturate following therapeutic APAP dosing.²³ Cimetidine

was proposed as an antidote for APAP poisoning, however it was supplanted by the more effective N-acetylcysteine.²⁴ To our knowledge, our study is the first to demonstrate the possibility of a drug vehicle being protective of APAP-hepatotoxicity in humans via manipulation of the P450 system.

The formation rate constants were equivalent for the phase-I metabolites between the solid and liquid arms (Figure 3). Since, for competitive antagonists such as PG, the same enzyme kinetics hold, just at different concentration ranges, equivalence of formation rate constants supports our hypothesis that PG was functioning as a CYP2E1 competitive inhibitor in the liquid group. This is also consistent with the dose-dependent protective effect of PG seen in mice.¹⁴

Limitations of our study include that we were unable to measure small concentrations of PG; we were unable to measure the decline in minor metabolites as they approached zero; and there was a difference in bioavailability between liquid and solid formulations. Since the PG concentration was 5% v/v in the preparation we administered, the peak predicted PG concentration would be 4.0 mg/dL, assuming an ideal instantaneous absorption, a specific gravity of PG of 1.04 g/mL; and a volume of distribution of 0.58 L/kg. This is very close to the mean PG concentration that we observed and helps explain why we were unable to detect propylene glycol in 6 of the 15 samples, as the limit of detection of the assay available to us was 5.0 mg/dL.

We could only observe our subjects for 9 hours and thus we were unable to follow the minor metabolite concentrations as they approached zero. We therefore had to apply a nonlinear regression model to extrapolate the concentrations out to infinity. Regardless, the measured plasma AUC's are consistent with the modeled AUC's; the measured results showed a 16-17% decrease in minor metabolite production in the liquid group and when extrapolated, this decrease is actually 25-28%.

Even though we administered equivalent liquid and solid doses (as close to 15mg/kg as possible), there was a trend toward greater bioavailability with the liquid formulation. As this absorption difference was not statistically significant, we did not correct our results and performed all analyses based on equal relative bioavailability. However, since absorption was greater in the liquid group, we would expect an increase in total metabolite formation, including P450 derived metabolites; the opposite was the case further strengthening our conclusions.

There are several important implications of our findings. First, we offer an alternative theory regarding the cause of decreased APAP toxicity seen in children. We propose that children tolerate APAP ingestion better than adults because they tend to ingest the liquid preparation, and are thus co-ingesting an inhibitor of the toxic bioactivation pathway. Previous theories have less well-grounded support. For example, infants and toddlers have a greater capacity for sulfonation, which is thought to be protective; however, glucuronidation capacity is decreased resulting in similar overall capacity for phase-II metabolism as older children and adults.^{8,11} The larger ratio of children's liver to total body size has been proposed to allow them to tolerate a greater milligram per kilogram APAP dose than adults, however no data

have been presented to support this.¹⁰ Our data suggests it is the preparation of drug, rather than the age of patient, which is responsible for the observed phenomenon of decreased susceptibility to acetaminophen poisoning in children.

More importantly, we propose that a less hepatotoxic formulation of APAP can be developed if co-formulated with a safe CYP2E1 inhibitor. Co-formulation with APAP to prevent toxicity is not a novel idea; a formulation with methionine has previously existed and a formulation with N-acetylcysteine has been proposed.²⁵ However, methionine itself has toxicity and N-acetylcysteine has a foul odor. Even though PG is a common excipient and generally considered safe, it might not be the ideal inhibitor to formulate with APAP. Our data suggest that higher PG dosing will be needed to achieve greater than 25% CYP2E1 inhibition; PG has well-defined toxicity when given intravenously²⁶ and likely has toxicity at higher oral dosing as well.²⁷

Finally, our study highlights the fact that pharmaceutical excipients can affect toxicity, pharmacokinetics or other aspects of drug effects. While this is unlikely to surprise members of the regulatory community or those working in the pharmaceutical industry, it is our impression that this is less frequently considered by clinicians and clinical investigators.²⁸

Conclusions

Ingestion of liquid relative to solid preparations of APAP in therapeutic doses results in a 16-28% decrease in the toxic CYP2E1 metabolites. This is likely due to the inhibition of CYP2E1 by PG, and may explain the decreased susceptibility of children to APAP toxicity. A less hepatotoxic formulation of APAP can potentially be developed if co-formulated with a CYP2E1 inhibitor.

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Figure 1. Metabolism of acetaminophen. A) P450 metabolism results in NAPQI radical production, which binds and reduces levels of intracellular glutathione leading to decreased redox buffering capacity. NAPQI arylated proteins and initiates toxicity. APAP CYP minor metabolites include APAP-glutathione, APAP-cysteinate and APAP-mercaptopurine. B) The compartmental model for acetaminophen metabolism used for the pharmacokinetic analysis.

Figure 2. Concentration-time curves for acetaminophen and metabolites. A) Acetaminophen B) Phase II metabolites C) CYP2E1 metabolites (Solid line represents solid preparation, dashed line represents liquid preparation. Error bars represent $\pm$ standard error)

Figure 3. Box-whisker plot of formation rate constants

Table 1

Results of plasma analysis (mean $\pm$ SE^a). AUC₀^t (zero to last observation) are trapezoidal measures and AUC₀[∞] (zero to infinity) are calculated by nonlinear regression.

	Solid	Liquid	p-Value ^b
Acetaminophen (APAP)			
Peak concentration (μg/mL)	15.4 ± 1.1	15.7 ± 1.1	0.811
Time to peak (minutes)	54.0 ± 7.9	40.0 ± 6.3	0.068
AUC ₀ ^t (μg.hr/mL)	51.4 ± 3.2	54.5 ± 3.5	0.089
AUC ₀ [∞] (μg.hr/mL)	59.6 ± 4.1	64.1 ± 4.8	0.143
APAP-glucuronide			
Peak concentration (μg/mL)	19.7 ± 1.0	19.2 ± 1.3	0.528
AUC ₀ ^t (μg.hr/mL)	107.3 ± 6.3	109.1 ± 7.2	0.648
AUC ₀ [∞] (μg.hr/mL)	157.2 ± 10.7	158.1 ± 9.6	0.922
APAP-sulfate			
Peak concentration (μg/mL)	6.20 ± 0.3	6.50 ± 0.5	0.266
AUC ₀ ^t (μg.hr/mL)	32.0 ± 1.5	35.1 ± 2.6	0.077
AUC ₀ [∞] (μg.hr/mL)	45.5 ± 2.8	51.2 ± 4.51	0.066
APAP-cysteinate			
Peak concentration (μg/mL)	0.42 ± 0.03	0.34 ± 0.03	0.003 ^c
AUC ₀ ^t (μg.hr/mL)	2.41 ± 0.21	2.01 ± 0.17	0.007 ^c
AUC ₀ [∞] (μg.hr/mL)	5.70 ± 0.77	4.29 ± 0.48	0.007 ^c
APAP-mercaptopurine			
Peak concentration (μg/mL)	0.136 ± 0.01	0.116 ± 0.01	0.016 ^c
AUC ₀ ^t (μg.hr/mL)	0.716 ± 0.07	0.602 ± 0.06	0.006 ^c
AUC ₀ [∞] (μg.hr/mL)	1.51 ± 0.18	1.09 ± 0.11	0.005 ^c

^a SE = standard error

^b Paired sample T-test, 2-tail

^c Statistically significant at p=0.05